BIOPOLYMER COMPOSITIONS FOR ECOLOGICAL PROTECTION AND GROWTH STIMULATION OF PLANTS

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Abstract
Presented herein are investigations in the preparation of an advanced , human-and environment-friendly plant protection composition based on selected bioactive biopolymers of the polysaccharide family. In the Institute of Biopolymers and Chemical Fibres the biopolymers were prepared and the biological activity of chitosan in the form of salt and gel and of hemicelluloses (galactoglucomannans) and of their compositions was tentatively evaluated by way of plate tests. Estimated was the impact of such preparations upon the stimulation of the germination rate of radish seeds at concentration of 0.1, 0.01 and 0.005% after 72 hours. The efficacy of the preparations was evaluated based on the number of germinated seeds in the green mass of the sprouts and their length in comparison to a reference in water at pH = 7.0. In Research Institute of Horticulture, Skierniewice, Poland the usefulness of selected biopolymer preparations was evaluated as agents to protect decorative plants against some pathogens (on leaves, in soil) such as Phytophtora cryptogea causing decay of shoots and roots. It could be found that the biopolymer compositions applied to the soil provided a much better rooting of chrysanthemum cuttings in a Phytophtora cryptogea - infected soil. The cuttings were growing much faster and the number of infected ones was largely reduced. The usefulness of a number of chitosan formulations was also evaluated for seed pickling. It was found that the formulations exerted a distinctly positive impact upon the germination, growth and health of the seedlings. Laboratory and greenhouse testing was made in the Institute of Plant Protection, Poznań, Poland where the biological activity of some biopolymer compositions was evaluated. A first selection of the biopolymers was done based on an in vitro examination of the impact upon bacteria growth. (Gram negative - Erwinia amylovora and Gram positive Clavibacter michiganensis) on agar or agarose plates and upon the generation of necrotic stains on beans and tobacco caused by lucerne mosaic virus and tobacco mosaic virus respectively. It was found that the preparations with original biopolymer concentration had not affected the viruses directly but stimulated the plants' immunity against the pathogens.

Key words: *polysaccharides, biopolymer compositions, plant protection, growth stimulation.*

1. Introduction

Synthetic polymers are presently being used all round the world for the protection of horticulture crops. Commercial plant protection agents are harmful to humans and pose a burden to the environment. They are not capable to jointly control a number of fungi and bacteria strains either. The pathogens acquire immunity from the chemicals after continual treatment. Drawbacks of the commercial pesticides lead toward a degradation of the environment and cause genetic changes in living organisms including humans. The disadvantages are a drawing force in the strive to natural plant protection agents like bio-preparations

The new generation of bio-preparations include i.a. elicitors which promote defense mechanisms and, in consequence, provide immunity of plants from many diseases caused by fungi and bacteria [1 - 5]. Components of the cell membrane of phytopathogenic fungi and plants like glucan, pectin, chitin and chitosan count to natural elicitors. The manufacture of commercial of elicitors from mushrooms is technically not feasible therefore natural polymers are sought available in bulk amounts and with properties similar to fungi- or bacteria-based ones. Chitin and chitosan are such candidates available in huge amounts from waste shells of crabs, shrimps and krill. Both substances give the chance of a wide use in agriculture.

Another group of promising biopolymers are galactoglucomannans (GGMs) from the polysaccharide family. GGMs are the most abundant in nature amongst the mannan group . Softwood holds about 20 - 25% of GGM. The backbone of GGM presents itself as a linear chain built up of β-D glucopyranose and β-D mannopyranose joined by β-1-4 glycoside bonds. Biological activity of GGM against plants has been documented. Some fractions of GGM isolated from fenngreek (*Trigonell*) and acetylated mannan from aloes applied in the form of gel on plant leaves have shown anti-viral and anti-bacterial activity [6, 7].

The preparation of polysaccharide- based plant protection agents harmless and save for humans and environment has been set up as a goal of the project. The biopolymers proposed in the project may constitute a class of plant care agents thanks to their bioactivity, unconventional action and harmlessness. The substances can find application whenever: (I) zero tolerance is imposed on conventional plant protection agents like fungicides (II) healthy food is produced in ecological farming (III) opposition is expressed toward GM food.

2. Materials and methods

2.1. Materials

The biopolymers and their compositions were prepared at Institute of Biopolymers and Chemical Fibres.

- Chitosan Delivered by Chemopol Co, Tada, India. Average molecular mass ($\bar{M}_v$) = 269.0 kDa, deacetylation degree (SD) = 78.0% , ash content = 60 ppm.
- Galactoglucomannans (GGMs),
- Derived from spruce wood,

- Lactic acid - pure for analysis, by Fluka Co,
- Potassium hydroxide - pure for analysis, by Fluka Co,
- Demi water,
- Radish seeds 'Lidka' - delivered by PNOS Co, Ożarów Mazowieki, Poland.

2.2. Preparation of chitosan salt

Chitosan lactate was obtained by reacting chitosan with a 0.5% wt. solution of lactic acid for 3 - 4 hours. After dissolution of the salt, a 1.0% its solution was obtained which was filtered through batiste cloth on a Bűchner funnel. Chitosan lactate was characterized by pH = 4.82 - 5.21, polymer content of 1.00% , $\bar{M}_v$ = 203.0 kDa and SD = 78%.

2.3. Preparation of chitosan salt gel

A 10% solution of potassium hydroxide was slowly poured to an adequate amount of 1.0% chitosan lactate solution to arrive at pH of 6.56 - 6.63 with polymer content in the range of 0.95 - 0.99%. Characteristic of the chitosan lactate gel: pH = 6.56 - 6.63, polymer content = 0.95 - 0.99 , $\bar{M}_v$ = 212.0 kDa, SD = 78%.

2.4. Hydrolysis of galactoglucomannans (GGMs)

The GGMs were obtained from spruce sawdust which were first ground in a Körner grinder and then boiled in a metal reactor at 80 - 120 ^{o}C for 90 minutes and a module of 4 : 1. The obtained filtrate was centrifuged, filtered through a glass funnel (porosity of 0.2μm) and freeze-dried in the laboratory lyophilizing cabinet ALFA 1-4, Christ Co. Content of saccharides was estimated by gas chromatography with following results: 1 g of the preparation contained 59.3 mg of mannose, 23.97mg of glucose and 55.7 mg of galactose.

2.5. Preparation of biopolymer compositions

To a solution of chitosan lactate or chitosan gel a solution of galactoglucomannan was added in amount of 5% wt. on chitosan content. The obtained composition was homogenized at controlled pH by stirring with a paddle agitator.

2.6. Estimation of the germination rate of radish seeds

An introductory estimation of the biological activity of selected chitosan forms (salt, gel), galactoglucomannans and compositions thereof was done based their impact upon the germination rate of radish seeds. To this end, circular filter papers holding 20 seed were placed on Petrie plates with 9 cm dia and soaked with 10 ml. of aqueous solutions of the polymer with concentration of 0.1, 0.01 and 0.005%. The impact of the preparations upon the germination rate was estimated after72 hours based on number of germinated seeds, their green mass and length in comparison with a reference in demi water at pH = 7.0.

2.7. Estimation of the anti-bacterial activity of biopolymer compositions

Estimation of the impact of the preparations upon the induction and development of hypersensitivity reactions on tobacco leaves (Nicotiana tabacum var. Xanthi) - Institute of Plant Protection – PIB, Poznań

Following pathogens were used in the examination of the impact of the preparation on the development of hypersensitivity reactions on tobacco leaves *(Nicotiana tabacum var. Xanthi):*

- *Erwinia amylovora* – isolate K3,
- *Pseudomonas syringae pv.phaseolicola* - isolate 560.

The bacterial inoculum was made up of an aqueous suspension of a 24 hours culture of *E. amylovora and P. syringae* pv *phaseolicola* with a density of 1 in McFarland scale.

Zero reference – distilled sterile water (DSW) injected to the plants.

Negative reference- the tested preparations diluted with DSW in the proportion of 1:10 injected to tobacco plants.

Positive reference – plants inoculated with an aqueous suspension of the bacteria *E.amylovora and P. syringae* pv *phaseolicola* with a density of 1 McF (equivalent to cell concentration of 10^6 jtk/ml).

Two methods were applied in the evaluation : direct action of the preparations on the bacteria and immunization. The tested preparation were, in the first method, diluted (1:10) with a bacteria suspension at concentration of 1 McF, and such suspension was injected to tobacco leaves in such a way that the injected area was lodged between two nerves of the leave. Presence or absence of the hypersensitivity reaction was observed after 48 hours . In the latter method, the tested preparations were, in a 1:10 dilution, injected to the intercellular space of the tobacco leaves in such a way that the injected area was lodged between two nerves of the leave. After 48 hours , bacteria suspensions were injected to the same places of tobacco leaves and the appearance and intensification of hypersensitivity reactions was observed.

2.8. Estimation of the antiviral activity of the biopolymer compositions- Institute of Plant Protection – PIB, Poznań

The examination was accomplished according to two model systems - plant- virus between which a hypersensitivity reaction proceeds reflected in the appearance of local necrotic stains:

- Bean / OL V1 (Olive latent Virus 1),
- Bean/ PSV (Peanut stunt Virus) – raises a systemic infection on the plant marked by the dying of plant's upper part.

Plant juice for infections (inoculum) was prepared from plants revealing viral infection (2 weeks after infection) by grinding the leaves in distilled water in the proportion of 1:5 followed by filtration through a double layer of mill gauze.

Two method were applied in the evaluation. In the first one, the sensitivity of bean to the tested biopolymers was estimated as well as efficacy of the biopolymers. These were used in aqueous suspensions in 10-, 50-, and 100- fold dilution of the initial preparations.

The aim of the second method was to proof that the anti-viral activity of the biopolymers consists in the induction of immunity to the bean. In the experiments a direct contact of the biopolymer with the virus was avoided thus eliminating its impact upon the virus. It could be accomplished by two ways : (1) the lower surface of the leave was treated with the biopolymer and three days later the upper surface of the same leave was infected with the virus, (2) the plant lower leaves were treated with the biopolymer and four days later the upper leaves (above the lower ones) were infected with the virus. The reference was made the same way while water was used instead of the biopolymer. For the Bean /OLV1, model the induced immunity was estimated based on the amount of necrotic stains on the treated- and reference plants.

2.9. Estimation of the efficacy of the different formulations of chitosan for uses as seed dressing - Research Institute of Horticulture, Skierniewice

Impatiens (*Impatiens valeriana*) seeds were used in the testing. These were dressed with selected biopolymer compositions in concentration of 1.0 and 2.0%. Biochikol 020 PC was used as a standard agent in concentration of 1.0 and 2.0%. Seeds first soaked in water and then immersed for 15 minutes in the solutions of the tested chitosan formulations were taken as reference. They were put onto four wet layers of sterile filtration paper on Petrie plates (dia 195 mm). The plates were incubated in darkness for 8 days at 22 - 24 ^{o}C. Random block mode was adopted in the experiments in 4 repetitions, 50 seeds each. The results were statistically analyzed. Observed was the amount of seed sprouts, length of the main root and number of side roots of the sprouts. The number of infected sprouts was counted and fungi settled on the sprouts were isolated. Following pathogenic fungi strains were used in the experiments: *Alternaria alternata* Ness.; *Botrytis cinerea* Pers.*; Colletotrichum gloeosporioides (*Penz.*) Sacc.; Fusarium avenaceum* (Cda) *Sacc.; Fusarium oxysporum* Schlecht.*; Fusarium solani* (Mart.) *Sny et Hans.*

2.10. Estimation of the impact of the biopolymer composition upon the development of phytophtorose on chrysanthemum - Research Institute of Horticulture, Skierniewice

Non-rooted seedlings were rooted in a turf substrate infected with Phytophthora Cryptogea. The plants were then placed on a shelf in the greenhouse and watered with selected biopolymer compositions. As reference seedlings were taken rooted in non- infected and infected medium without protection. Biochikol 020 PC in concentration of 1.0% was used as standard agent. The pots were next covered with a foil and the seedlings were rooted for 14 days at 18 - 23 °C. After that time the amount of dead seedlings and rooted plants was counted and plant height measured. Random block mode was adopted in the experiments in four repetitions, 5 seedlings each. It was repeated after 2 weeks.

3. Results and comments

A main objective of the investigation was to prepare a modern plant protection agent safe and friendly to the environment based on selected biopolymers derived from renewable resources. The authors' intention is to bring forth a preparation that could in the future be used in ecological farming and in the production of healthy food.

3.1. Estimation of the biological activity of biopolymer composition upon germination rate of radish seeds - Plate tests at Institute of Biopolymers and Chemical Fibres

At this stage of the investigation, some biopolymer preparations were evaluated (characteristic in ***Table 1***) enabling a selection of candidates showing highest ability to stimulate the germination of radish seeds. The activity of preparations used in two repetitions at concentrations of 0.1, 0.01 and 0.005% was estimated after 72 hours. The efficacy was defined on base of amount of germinated seeds, green mass of the sprouts and their length in comparison with a reference (water, pH = 7.0). In Results of the testing of selected biopolymer preparations are shown in ***Tables 2*** and ***3***. while in ***Figure 1*** a photographic documentation can be seen of the testing.

Table 1. *Characteristic of biopolymer preparations used in the tests on plants.*

Symbol of the preparation	Concentration of the polymer, %	pH
ML/3/G71	1.00	4.82 - 5.21
ML/3/G71 + 5% GGM	0.95 - 0.97	4.82 - 5.16
ML/4M/G71	0.95 - 0.98	6.56 - 6.63
ML/4M/G71 + 5% GGM	0.93 - 0.95	6.56 - 6.63

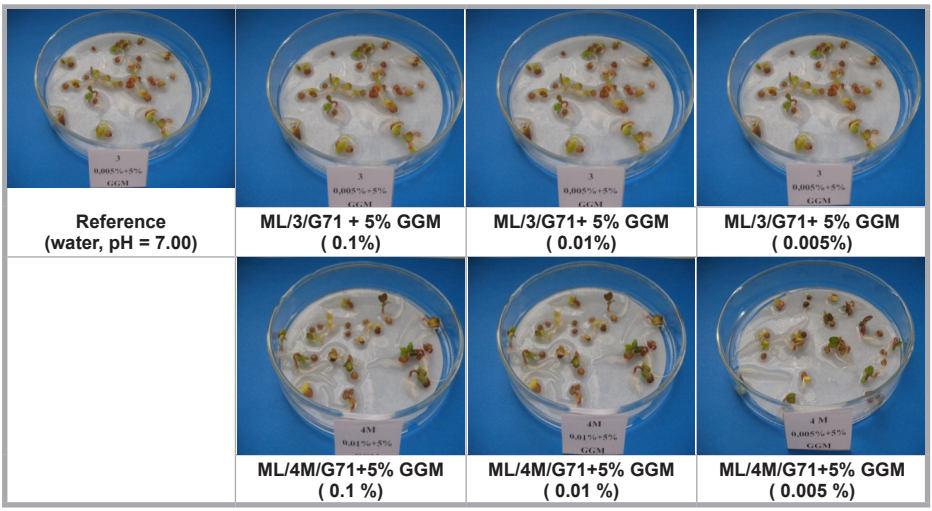
Table 2. *Impact of selected chitosan lactate preparations on the germination rate of radish seeds.*

Symbol of the preparation	Concentration, %	pH	Number of sprouts		Mass of the sprouts, g		Length of the sprouts, cm	
			average	% of reference	average	% of reference	Average of two repetitions	% of reference
Reference	-	7.00	18.0	100.0	1.0373	100.0	2.79	100.0
ML/3/G-71	0.1	5.05	18.0	100.0	1.0321	99.5	1.67	59.9
	0.01	6.18	19.5	**108.3**	1.2835	**123.7**	3.13	**112.0**
	0.005	6.63	20.0	**111.1**	1.3111	**126.4**	4.03	**144.4**
ML/4M/G-71	0.1	6.23	19.5	**108.3**	1.0847	**104.6**	2.35	84.1
	0.01	6.96	20.0	**111.1**	1.1686	**112.7**	2.99	**107.2**
	0.005	7.00	20.0	**111.1**	1.4195	**136.8**	3.22	**115.4**

Table 3. *Impact of selected biopolymer compositions upon the germination rate of radish seeds.*

Symbol of the preparation	Concentration %	pH	Number of sprouts		Mass of the sprouts, g		Length of the sprouts, cm	
			average	% of reference	average	% of reference	average	% of reference
Reference	-	7.00	18.5	100.0	0.7038	100.0	1.21	100.0
ML/3/G71+ 5 % GGM	0.1	4.90	17.5	94.6	0.7533	107.0	2.23	**184.3**
	0.01	5.94	17.5	94.6	0.6687	95.0	1.53	**126.4**
	0.005	6.79	20.0	108.1	0.8859	125.9	1.83	**150.8**
ML/4M/G71	0.1	6.23	19.5	108.3	1.0847	104.6	2.35	**84.1**
	0.01	6.96	20.0	111.1	1.1686	112.7	2.99	**107.2**
	0.005	7.00	20.0	111.1	1.4195	136.8	3.22	**115.4**
ML/4M/ G71+ 5% GGM	0.1	5.97	20.0	108.1	0.8511	120.9	1.79	**147.5**
	0.01	6.56	18.5	100.0	0.8378	119.0	2.23	**184.3**
	0.005	6.84	18.5	100.0	0.8694	123.5	2.51	**207.4**

Figure 1. *The test of stimulating germination rate of radish seeds.*



It was found that chitosan lactate with different pH stimulates the germination of radish seeds contributing mainly to mass increase of the sprouts, and to their length.

From the results, the conclusion can be drawn that the tested biopolymer compositions enhance the germination of radish seeds adding mainly to the length growth of the sprouts. The preparation marked ML/4M/G71+5% GGM revealed best results with increase of the sprouts length growth exceeding 100% at only 0.005 % of concentration.

3.2. Estimation of the impact of the preparations upon inducement and development of hypersensitivity reactions on tobacco leaves (*Nicotiana tabacum* var. Xanthi - Institute of Plant Protection – PIB, Poznań)

It could be found that on tobacco plants, pretreated with the tested preparations, hypersensitivity reactions, induced by the injection of bacteria *P. syringae pv. Phaseolicola,* were fully blocked. (Method 1). The effect was manifested for all three tested preparations (***Figures 2, 3*** and ***4***). The effect was not as distinct when bacteria *E. amylovora* were used (***Figures 5, 6*** and ***7***).

In attempts to immunize tobacco plants by the action of the examined preparation (Method 2), a distinct restraint was observed of both appearance and intensification of the hypersensitivity reaction when compared to the positive reference. (***Figures 8, 9*** and ***10***). At the same time disease symptoms were also restrained with all 3 tested preparations, however, in relation to *P. syringae* pv *phaseolicola* only . (***Figures 8, 9 a***nd ***10***). The effect was absent in case of *E. amylovora* (***Figures 11, 12*** and ***13***).

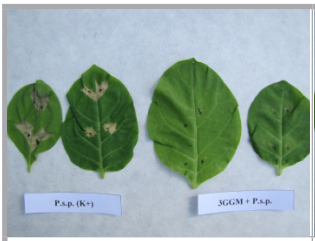


Figure 2.

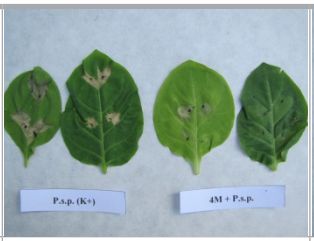


Figure 3.

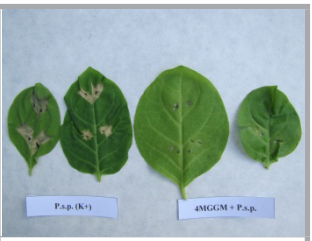


Figure 4.

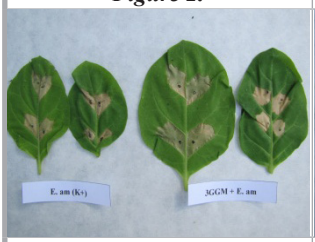


Figure 5.

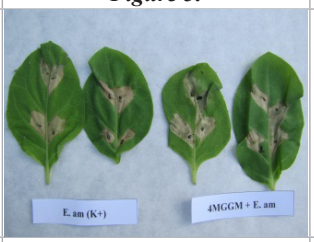
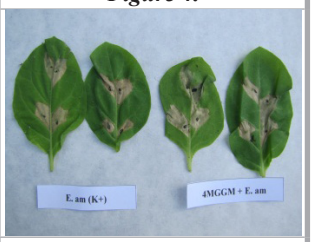


Figure 6.



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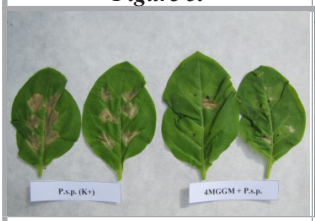

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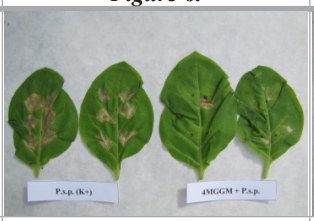

Figure 9.

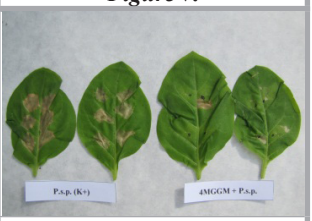

Figure 10.

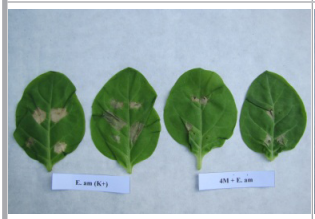

Figure 11.

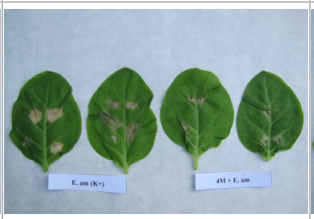

Figure 12

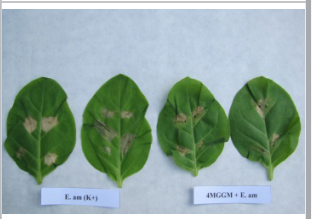

Figure 13.

The restraint of production or bonding and blockage of the mentioned elicitors, produced by pathogens and needed to induce hypersensitivity, is a supposed reason of inhibiting the hypersensitivity reaction in tobacco plants.

In the tests in which the examined preparation only was injected to the plant and the bacteria only after 48 hours , the concentration of the preparation decreased at the injection point caused by diffusion and bonding with particles of the plant tissue. The result was an only partial inhibition of the hypersensitivity reaction.

The selective action of the examined preparations in relation only to *P. syringae* pv *phaseolicola* and lack of any inhibition effect with of *E. amylovora* can be explained with

Figure 14. *Direct reaction of bean leaves to the sprinkling with polymers ML/4M/G71 + 5% GGM. Leaves sprinkled (from left to right) with 10×, 50× and 100× dilution of the preparation.*

Figure 15. *Direct reaction of bean leaves to the sprinkling with polymers ML/3/G71 + 5% GGM. Leaves sprinkled (from left to right) with 10×, 50× and 100× dilution of the preparation.*

Figure 16. *Impact of the polymers upon systemic infection by the Peanut stunt Virus. Left – decaying plants sprinkled with water before infection. Pot on the right- plants before infection sprinkled with ML/4M/G71 + 5% GGM at concentration of 0.019%*

that the bacteria belong to quite different families *Pseudomonace* and *Enterobacteriace* respectively and an allegedly resulting different LPS chemical composition. The importance of the factor has been described elsewhere [9, 10].

3.3. Estimation of the sensitivity of bean plants to biopolymers and their efficacy – Institute of Plant Protection - PIB, Poznań

The conducted trials have shown that bean is exceptionally susceptible to the tested polymer compositions which is manifested in two ways : (1) direct reaction of the plant and (2) ability of the plant to develop an immunity reaction. Carefully sprinkled with aqueous solutions of the polymers in 10-, 50-, and 100-fold dilution, bean plants reacted with the appearance of tiny necrosis stains as soon as after 2 days after the treatment. Intensity of the appearance of necrotic stains was decreasing with higher dilution of the polymer and was still observed at an even 100-fold dilution (***Figures 14, 15*** and ***16***).

Even at as low as 0.0093 to 0.0098 % concentration, the polymers manifested a high restraining effect in relation to local viral infection induced by OLV 1 (Table 4.). The effect is also transmitted to the systemic infection in the system bean and Peanut stunt virus . The biopolymer- treated plants did not decay after the infection (Figure 16). The remaining tested biopolymers revealed a similar effect.

3.4. Biopolymer- Induced immunity against viral infection in bean plants – Institute of Plant Protection - PIB, Poznań

Based on earlier experiments, it may be concluded that polymers do not affect directly the infectiousness of viruses. The conclusion is confirmed with that a blend of virus and polymer induces infection in tobacco while the same blend does not in bean. The explanation is that the lack of infection in bean was not caused by the inhibition of virus infectiousness but resulted from the blockage by the polymer of receptors specific to the virus ap-

Table 4. *Impact of the biopolymers upon the infection of bean with Olive latent Virus 1; *in brackets – the final concentration of polymer in the aqueous solution of the preparation.*

Preparation	Average number of stains/ leave	Inhibtion of the infection, %
Reference (H_20)	167.2	
ML/3/G71+ 5% GGM (0.0095%)*	38.2	77.2
ML/4M/G71 (0.0098%)	2.0	98.8
ML/4M/G71+ 5% GGM (0.0093%)*	10.4	93.8

Table 5. *Induction of immunity on non- treated surfaces (upper) of a treated leave (lower surface).*

Preparations	Average number of stains /leave	Inhibition of infection, %
Reference (H_20)	439	
ML/3/G71+GGM	4.4	99.0
ML/4M/G71	5.7	98.7
ML/4M/G71+GGM	3.4	99.2

Table 6. *Induction of immunity on non- treated leaves (upper) of treated bean plants (lower leaves).*

Preparations	Average number of stains /leave	Inhibition of infection, %
Reference (H_20)	147.1	
ML/3/G71+GGM	68.5	54.5
ML/4M/G71	83.2	43.5
ML/4M/G71+GGM	92.4	39.2

pearing on cell membranes ,and, to a certain degree, from induced immunity. Two different experiments in which the virus was in no contact with the polymers indicate that they induce immunity to the bean against infection –not related to the blocking of receptors but to the inhibition of virus growth. The shorter the distance between the treated and infected parts of the plant the higher is the level of induced immunity. Inhibition was close to 100% when the lower part of a leave was treated with the biopolymers and the upper part of the same leave was infected with the virus. (***Table 5***). The inhibition effect was much lower when the low leaves of the plant were polymer-treated and the upper level leaves were infected (***Table 6***).

Concentration of the polymer in used solution was in the range of 0.019 do 0.020%

To sum up: the results lead to the conclusion that the antiviral action of the biopolymers on bean comprises two phenomena (1) blockage of receptors specific to the virus resulting from the affinity of the polymers to the receptors (2) induction of immunity.

3.5. Evaluation of effectiveness of various polymer compositions in the use as seed dressing – Research Institute of Horticulture, Skierniewice

Biopolymer preparations specified in ***Table 1*** were used in the research concerning seed dressing. All employed formulations of the polymers regardless their concentration caused a much better seed germination. In case of impatiens seeds, the highest amount of germinated ones appeared after a dressing with the material marked ML/4M/G71 and ML/4M/ G71 + 5% GGM (***Table 7, Figure 17***). The dressing protected the seedlings against *Alternaria alternata and Botrytis cinerea,* detected on reference plants and seedlings obtained from seeds dressed with the commercial Biochikol 020 PC (***Tables 7 & 8***). Roots were twice longer after the dressing with polymer composition ML/4M/G71. All tested polymer preparations showed a much higher effectiveness in comparison with Biochikol 020 PC.

Table 7. *Impact of concentration and type of the bio-composition upon germination, development and health of impatiens valeriana seedlings after 10 days from seeding.*

Preparation	Number of germinated seeds (n=50)	Number of disease-affected seedlings d (n=50)	Length of roots, mm
1. Reference	18.5 a	2.5 b	4.6 a
2. Ml/3/ G71 + 5% GGM 1.0%	28.5 b	0 a	6.5 a-c
3. ML/3/ G71 + 5% GGM 2.0%	13.5 a	0 a	5.4 ab
4. ML/4M/ G71 1.0%	37.8 c	0 a	8.4 d
5. ML/4M/ G71 2.0%	40.5 c	0 a	7.6 cd
6. ML/4M/ G71 + 5% GGM 1.0%.	37.0 bc	0 a	6.9 b-d
7. ML/4M/ G71 + 5% GGM 2.0%	37.0 bc	0 a	6.1 a-c
8. Biochikol 020 PC 1.0%	16.0 a	2,0 b	4.4 a
9. Biochikol 020 PC 2.0%	13.3 a	1.8 b	5.4 ab

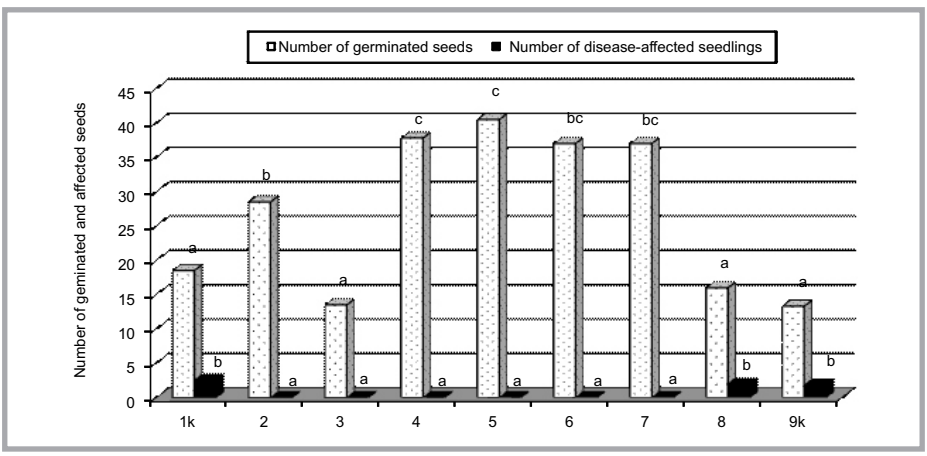

Figure 17. *Dependence of germination and health condition of impatiens seeds (Impatiens waleriana) upon the concentration of various biopolymer formulations used for seed dressing; 1. Refernce; 2. ML/3/ G71 + 5% GGM 1.0%; 3. ML/3/ G71 + 5% GGM 2.0%; 4. ML/4M/ G71 1.0%; 5. ML/4M/ G71 2.0%; 6. ML/4M/ G71 + 5% GGM 1.0%; 7. ML/4M/ G71 + 5% GGM 2.0%; 8. Biochikol 020 PC 1.0%; 9. Biochikol 020 PC 2.0%.*

3.6. Estimation of the impact of biopolymer compositions upon the development of phytophthorose on chrysanthemum – Research Institute of Horticulture, Skierniewice

Non rooted seedlings of chrysanthemum were rooted in a turf substrate artificially infected with *Phytophthora cryptogea* and, next, watered with the examined biopolymers. After 14 days, an estimation was made of the amount and height of the rooted chrysanthemum seedlings. Results are presented in ***Table 8***.

Table 8. *Impact of biopolymer compositions upon the amount and height of the rooted plants.*

Preparation	Number of dead seedlings n = 5	Number of rooted seedlings n = 5	Average height of seedlings in mm after 14 days
Reference non infected	0a	5.0 c	93.4 b
Reference infected	1.8 b	2.5 a	83.7 a
Biochikol 1%	0.8 b	4.3 bc	102.1 c
ML/3/G71+ 5 % GGM 0.5%	1.0 ab	3.5 b	98.1 bc
ML/3/G71+ 5 % GGM 0.1%	0.8 ab	4.3 bc	99.1 c
ML/4M/G71 0.5%	0.8 ab	4.0 b	98.8 c
ML/4M/G71 0.1%	0.5 a	4.3 bc	101.5 c
ML/4M/G71+ 5 % GGM 0.5%	0.5 a	4.0 b	101.9 c
ML/4M/G71+ 5 % GGM 0.1%	0.8 ab	4.0 b	100.4 c

Phytophthora cryptogea is nowadays a serious pathogenic factor to decorative plants in nursery and greenhouse planting causing decay of the shoots' base [11 - 14]. The application of the examined biopolymer composition resulted in a slight reduction of the necrosis in chrysanthemum seedlings, and in their much better rooting (***Table 8***). Most of the composition caused a distinctly faster growth of the rooted seedling in comparison with untreated ones. No major differences could be seen in the effectiveness in dependence on the concentration of the used preparation.

4. Conclusions

- The tested biopolymer composition stimulate germination of radish. Best results were achieved with preparations containing 5% wt. of galactoglucomannans.
- The tested bio-preparations in the original polymer concentration do not directly affect infectiousness of viral particles.
- It was documented that the antiviral action of the tested preparations depends rather on the type of plant than on the type of the virus.
- Two phenomena may contribute to the antiviral action of the bio-polymers on bean plants: (1) blockage of receptors specific for viruses resulting from the affinity of the polymers to the receptors (2) induction of immunity.
- A positive impact was found of the tested biopolymer compositions upon germination of seeds ,and growth and health of the seedlings.
- All tested compositions imposd a total protection to impatiens plants against *Alternaria alternata* and *Botrytis cinerea*

- Obtained results indicate that formulations of chitosan at concentration of 1 – 2.0% can be used as plant protection and growth stimulating agents.
- The tested biopolymer compositions when used to soil were causing a distinctly better rooting of chrysanthemum seedlings in a medium infected with *Phytophthora cryptogea* and a much faster growth.

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